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Application of Cavitation Promoting Surfaces in Management of Acute Ischemic Stroke

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Abstract

High frequency, low intensity ultrasound has the potential to accelerate the clearance of thrombotic occlusion in the absence of cavitation. At high frequency ultrasound, high acoustic pressures, > 5.2 MPa, are required to generate cavitation in thrombus. The focus of this study was to reduce the cavitation threshold by applying materials with appropriate nucleation sites at the transducer-thrombus boundary to further augment sonothrombolysis.

Heterogeneous and homogenous nucleation sites were generated on the outer surface of a polyimide tube (PI) using microfringed (MPI) and laser induced (LPI) microcavities. The cavitation threshold of these materials was determined using a passive cavitation detection system. Furthermore, the biological impact of both materials was investigated *in vitro*. The results revealed that both MPI and LPI have the potential to induce cavitation at acoustic pressure levels as low as 2.3 MPa. In the presence of cavitation, thrombolysis rate could be enhanced by up to 2 times without any evidence of hemolysis that is generally associated with cavitation activities in blood.

A prototype ultrasonic catheter operating at 1.7MHz frequency and acoustic pressure of 2.3MPa with either of MPI or LPI could be considered as a viable option for treatment of acute ischemic stroke.

Keywords

SonoThrombolysis; Intra-Arterial treatment; Acute Ischemic Stroke; Cavitation; Hemolysis; microcavities

INTRODUCTION

There are two main types of stroke: 1) *Ischemic Stroke*, caused by a thrombus blocking the blood supply and 2) *Hemorrhagic Stroke*, caused by the rupture of a blood vessel. Ischemic stroke is the most common type accounting for 83% of all stroke incidents in the United States. The damage due to stroke depends on the location and size of the brain area deprived of blood and oxygen. In ischemic stroke the sooner the blood supply is restored, the better are the patient's chances of survival with minimum or no brain tissue damage [1]. Currently, recombinant tissue Plasminogen Activator (rt-PA) is the only drug that is used for dissolving blood clots in the brain and has shown positive outcomes when this drug is administered

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intravenously to the patient within 3–4.5 hours of onset of stroke symptoms [2, 3]. Emerging treatments of ischemic stroke are focusing on faster reperfusion and neuroprotection. Intra-arterial treatment is among the emerging treatments that promise higher rates of recanalization and usage of lower dosage of thrombolytics compared with Intravenous therapy [4].

It is well-known that the thrombolytic activity of drugs is enhanced in the presence of ultrasound (US) [5–7]. Microsonic catheter (EKOS Corporation, Bothell, Washington) designed for intra-arterial therapy in neurovasculature allows for local delivery of thrombolytic drug in the presence of a high-frequency, low intensity US field. *In vitro* studies have demonstrated that low intensity acoustic output of this catheter increases enzyme mediated thrombolysis in the absence of cavitation [8]. Additional studies demonstrated that addition of extraneous US contrast agents to the treatment modality with Microsonic catheter generated acoustic emissions indicative of cavitation and augmented the thrombolysis rate even further [8, 9].

The nucleation of gas bubbles from cavities in a surface in contact with a liquid is a widely recognized phenomenon. This process has been extensively analyzed by mathematical, thermodynamic and physiological modeling in the past two decades [10–14]. Hence, the possibility of these surface cavities generating an intrinsic source of nuclei and hence lowering the acoustic pressure threshold for inducing cavitation to further augment the sonothrombolysis treatment with Microsonic catheter became the focus of this investigation.

A cavitation-promoting material should provide bubble nucleation sites on its surface that can be activated when an appropriate level of oscillating mechanical pressure is present. A hydrophobic material is an ideal candidate for this application because water does not uniformly “wet” a hydrophobic material. When immersed in water, small nanobubbles coat the material's surface [15]. In general, a material's level of hydrophobicity is determined by its chemical composition: hydrophobic materials lack the active groups capable of forming hydrogen bonds with water. In addition to the surface chemistry, however, the surface texture also contributes to the overall level of hydrophobicity. In fact, special surfaces, termed superhydrophobic surfaces, have been developed in which the hydrophobicity is greatly enhanced by adding micron-scale surface structure [16]. In addition to enhancing hydrophobicity, a material's surface texture may also contribute to bubble nucleation sites in the form of small pockets of gas trapped in crevices. This idea, first proposed by Harvey *et al.*, says that a submicroscopic gas body will be stabilized against surface-tension-driven diffusion if it exists in a crevice or crack of suitable shape in a solid surface [17]. Thus, the surface structure of a material may contribute to the material's cavitation-promoting ability by increasing hydrophobicity, or providing sites for trapped gas, or both.

In this study, the ability of promoting cavitation of two microstructures with heterogenous and homogenous nucleation sites was investigated. Experiments were designed to record and analyze cavitation activity in real time during sonothrombolysis. Efforts were made to quantify two types of cavitation, inertial and stable, to identify the dominant type of cavitation in this application. Furthermore, clinical application of these materials in sonothrombolysis setting was explored by determining the bioefficacy impact. Since cavitation is also known to be associated with adverse biological effects such as hemolysis [18], hemorrhage [19] and microvasculature damage [20], the only clinical safety issue that could have been explored *in vitro*, namely hemolysis, was investigated as well.

METHODS

Selected Microstructures

A PI tube is currently used as the solid hydrophobic material interface between the ultrasound transducer of a Microsonic catheter and blood clots. PI is a popular material used in the design of short term implanted medical devices. These tubes had a 0.86 mm internal diameter with a 0.051 mm wall thickness (Accellent; MA, USA).

The first approach to reduce the cavitation threshold was increasing the hydrophobicity of PI by coating the outer surface with materials of lower surface tensions. However, application of a thin thickness, 0.076 mm, of materials such as Parylene or Teflon did not reduce the cavitation threshold (data not shown). The next approach was generating microcavities on the outer surface of the PI tubes. Two types of microcavities were investigated in this study, microfringe and laser induced.

Heterogeneous nucleation sites: Microfringe Texture—Random nucleation sites were produced on the outer surface of PI tube by using 600-grit sandpaper manually. These microcavities were generated by different operators and not in the exact same manner. Figures 1a and 1b show microscopic images of the surfaces of the two sample tubes, plain polyimide (PPI) and polyimide with microfringe texture (MPI).

Homogenous nucleation sites: Laser Induced Texture—A mask projection technique with Excimer laser was utilized for this purpose. The depth of a laser-drilled blind hole was calibrated versus number of laser pulses used at constant fluence and wavelength using surface profilometry (data not shown). Each PI tube was individually ablated with one out of 9 specific blind hole (divot) patterns by Resonetics (Nashua, NH). Patterns were repeated on the external circumference of the tubing for specified length of 3 ± 10 mm starting from a 10 ± 0.10 mm from the distal tip of the PI tubing. Entrance diameter and depth are key feature characteristics. None of the features did penetrate entire thickness of tubing wall. Entrance diameters were 5, 10 and 15 microns and selected depths were 5, 10, and 18 microns. The repeated laser pulses generated a half angle taper of 3 to 5 degrees; hence the diameters at the bottom of the blind hole were smaller than entrance diameters. Among all patterns, the one with the entrance diameter of 10 microns and depth of 18 microns was the source of continuous generation of cavitation over longer period of time; hence this pattern was chosen for further investigation.

Prototypes Design

A Microsonic catheter was used to build prototypes by replacing the PPI sleeve around the transducer area with MPI or LPI. Figures 2a & 2b present an image of Microsonic catheter and its acoustic pressure field map, respectively. Between the tests, materials were wiped with 70% Isopropanol alcohol, and blow dried thoroughly (~20 sec) with canned air.

Acoustic parameters

The acoustic protocol known to accelerate thrombolysis in the absence of cavitation was named US_1 . The acoustic pressures at which the cavitation events were detectable was recorded as cavitation threshold for each material. The quantity of cavitation events was dependent on acoustic pressure level, pulse duration, pulse repetition factor, and material structure type. The acoustic protocol with minimum acoustic pressure required to generate continuous cavitation events from both MPI and LPI during sonothrombolysis was named US_2 . The details of both acoustic protocols, US_1 and US_2 , are described in Table 1.

Acoustic Sources

Microsonic catheters—A cylindrical non-focused piezoceramic transducer mounted along the distal tip and coaxial with the central lumen of the catheter was used for bioefficacy and hemolytic experiments. The nominal dimensions of the radiating cylindrical surface were 0.81mm outer diameter, 0.45mm inner diameter, and 2.1mm length. Due to the absence of an ultrasound transmission barrier built along the inner diameter of this cylindrical transducer, some inward ultrasound transmission is to be expected. The characteristic of this inner acoustic field can not be directly measured; however, as compared to 76% efficiency of outward radial transmission measured by a radiation force balance, it could be considered negligibly small.

A function generator (Agilent, Palo Alto, CA,) connected to a RF power amplifier (ENI Technology Inc., Rochester, NY) was used to drive the transducers at 1.7 MHz in pulsed mode. According to the type of PI sleeve used to cover the external surface of cylindrical transducers, the prototypes were divided into three groups: PPI, MPI, and LPI. Each one of these catheter prototypes were operated with two acoustic protocols. The first protocol, US₁, is the operating parameters used in the Interventional Management of Stroke (IMS II) clinical trials with a spatial-average acoustic pressure of 1.37 MPa. As demonstrated previously, this protocol in the presence of PPI sleeve enhances thrombolysis in the absence of cavitation [8]. The second protocol, US₂, is used to induce continuous cavitation events in sonothrombolysis setting with a spatial-average acoustic pressure of 2.32 MPa. To ensure that similar acoustic pressures were applied at each test, the transducers were tested by a radiation force balance prior to each test.

Focused transducer—The catheter prototypes could not be used in a passive cavitation detection set up for capturing cavitation signatures. To capture the cavitation signal during the sonothrombolysis, the catheter mounted transducer had to be placed in the middle of the clot for sonothrombolysis to be effective. In this way, the catheter transducer was placed within the focal point of hydrophone, and it allows the high magnitude first harmonic to be captured by hydrophone among other cavitation signals. Consequently, the tailing of high magnitude first harmonic of acoustic field emitted by catheter transducer overlapped with subharmonic signal. To resolve this issue, an attempt was made to aim the hydrophone few millimeters away from the catheter transducer. However, at the distance that the overlap of main signal with subharmonics would have been minimized, the magnitude of cavitation signals was significantly weakened and was hard to detect.

Hence, an external acoustic source was used for this test. The acoustic axis of external transducer was perpendicular to the acoustic axis of the hydrophone and faced an acoustic absorber at the end of opposite tank wall (Figure 3). This particular set up minimizes the magnitude and tailing of first harmonic of main signal detected by hydrophone. Consequently, it minimized the subharmonic signal overlap with the tailing of the main acoustic signal.

A spherically-focused (3.5 cm diameter; 5.5 cm radius of curvature) ultrasound transducer (Sonic Concepts, WA, USA) was used to generate an acoustic wave of precise pressure amplitude in the polyolefin sample tube. The transducer was positioned so that the focal zone was centered in the polyolefin test tube, both laterally and vertically. The -6 dB focal width and depth were 1.6mm and 17.6mm, respectively. The acoustic output parameters were matched the acoustic output of catheter mounted cylindrical transducers. The focused transducer was calibrated by conducting a daily measurement of electrical input versus acoustic output in degassed water. This transducer was used as the acoustic source to determine the cavitation threshold for the MPI and LPI surfaces.

Passive Cavitation Detection

The passive cavitation detection apparatus [21, 22] and data collection and evaluation were described previously [8] with the exception of using an acoustic source external to the test sample instead of a catheter-mounted ultrasound transducer placed in the middle of the test sample. The purpose of this test was to determine the acoustic pressure threshold required to induce cavitation for MPI and LPI. It was additionally intended to quantify the cavitation events occurring during sonothrombolysis in the presence of each PPI, MPI, and LPI tube immersed in the middle of human pooled whole blood clot when exposed to acoustic protocols of US₁ and US₂.

The thrombolytic drug, rt-PA, was delivered with the same concentration and flow rate used in *in vitro* thrombolysis study for the duration of 10 minutes. Each tube was placed in focal point of the external focused transducer. To limit the collected data to manageable file sizes, “snapshots” of noise activity were obtained by digitizing transient capture “bursts” of the hydrophone signal at six time points for each sample during the treatment: 0, 1, 2.5, 5, 7.5, 10 minutes. Each transient capture burst was timed to coincide with the exposure interval of the pulsed ultrasonic source. Each snapshot consisted of a digitized record of 50 consecutive bursts. The total duration of captured 300 bursts during the 10 minutes sonothrombolysis treatment time corresponds to 20 seconds. During each acquisition, a single ultrasound burst is recorded at a sampling rate of 50 MHz.

Data analysis consists of quantifying certain frequency components in the recorded signal that are characteristics of cavitation activity. The time-domain waveform is converted to the frequency domain using a 2048-point Fast Fourier Transform (FFT). The FFT amplitude at the subharmonic frequency is used to indicate stable cavitation, and the broadband noise integrated from 4 to 10 MHz is used to indicate inertial cavitation. The cylindrically focused, broadband, PVDF hydrophone used in this study had a flat frequency response to 10 MHz.

Degassed, 0.2 µm filtered water served as non-cavitating control in this experiment since the cavitation pressure threshold of this medium is typically about 3.0 MPa at low megahertz frequencies [23]. A cavitation threshold was defined as 4 times the standard deviation of the baseline noise amplitude for 1000 recorded baseline bursts. A true rise in broadband noise and subharmonics over the cavitation threshold was identified as peak subharmonics and broadband noise occurrences above cavitation threshold. The minimum number of samples in each test group was 5.

Thrombolysis Procedure

An *in vitro* flow model was utilized to evaluate the impact of MPI and LPI on sonothrombolysis under dynamic flow conditions. This test system, rt-PA preparation, clot formation, and thrombolysis procedure have been described in detail in a previous publication [24]. Briefly, human pooled citrated blood (Innovative Research, Southfield, MI) was formed into a clot by adding 0.2 ml of 0.25 M calcium chloride and 0.2 ml of 12.5 U/ml bovine thrombin to 2.0 mL of blood. Upon 10 min incubation at 37 C, the clot was removed from the tube, washed in saline and cut to 1 cm segments. Each clot was dried on 3 layers of filter paper for 20 seconds, rolling it over every 5 seconds and weighed on a precision balance prior to treatment. Then the clot was placed in one of the two parallel arms of the flow system that was submerged in a 37°C water bath.

A programmable hot plate (Thomas Scientific, Swedesboro, NJ) maintained the temperature of saline circulating through the system at 37 C upon reaching the bifurcation site as measured by an inline thermistor. A peristaltic pump (Cole-Parmer, Vernon Hills, IL) provided constant pulsating flow at 150 mL/h to the system. A pressure transducer

connected upstream of the bifurcation site allowed constant monitoring of the perfusion pressure in the flow loop. A metal pinch-tight tube clamp placed distal to the bifurcation site was used to define the flow resistance and maintain pressure between 20 and 25 mmHg in the loop. One of the prototype catheters was primed with rt-PA and placed in the clot. Subsequently, a dose of 0.3 mg/ml of rt-PA was delivered into the clot at a flow rate of 12 ml/h over 10 minute. Concurrent to the rt-PA delivery ultrasound was turned on for 10 min, during which time the catheter was advanced inside the clot in 2 mm increments every 2.5 minutes.

At the end of the 10 minute treatment period, the clot was removed from the tubing, washed in saline, dried and weighed. The clot weights recorded before and after treatment were used to calculate the percentage of weight loss which was used as measure of lysis. The percentage of clot weight loss for each treatment group is defined as:

$$\frac{W_b - W_a}{W_b} * 100 \quad (\text{Equation 1})$$

Where W_b and W_a are the average clot weight (mg) of any clot sample group before and after treatment, respectively. There were a total of 14 treatment groups in this experiment:

1. Clots treated with saline only: S
2. Clots treated with rt-PA only: T
3. Clots treated with saline plus US_1 and prototypes equipped with PPI: S+ US_1 +PPI
4. Clots treated with rt-PA plus US_1 and prototypes equipped with PPI: T+ US_1 +PPI
5. Clots treated with saline plus US_1 and prototypes equipped with MPI: S+ US_1 +MPI
6. Clots treated with rt-PA plus US_1 and prototypes equipped with MPI: T+ US_1 +MPI
7. Clots treated with saline plus US_1 and prototypes equipped with LPI: S+ US_1 +LPI
8. Clots treated with rt-PA plus US_1 and prototypes equipped with LPI: T+ US_1 +LPI
9. Clots treated with saline plus US_2 and prototypes equipped with PPI: S+ US_2 +PPI
10. Clots treated with rt-PA plus US_2 and prototypes equipped with PPI: T+ US_2 +PPI
11. Clots treated with saline plus US_2 and prototypes equipped with MPI: S+ US_2 +MPI
12. Clots treated with rt-PA plus US_2 and prototypes equipped with MPI: T+ US_2 +MPI
13. Clots treated with saline plus US_2 and prototypes equipped with LPI: S+ US_2 +LPI
14. Clots treated with rt-PA plus US_2 and prototypes equipped with LPI: T+ US_2 +LPI

A minimum of five data points were generated for each treatment group. Statistical analysis was performed using Student's T-test and One-Way ANOVA at a 0.05 level of significance.

Hemolysis Procedure

It is well known that cavitation activity in the blood could cause hemolysis [25–27]. Hemolysis is a potential risk factor for mortality and life-threatening clinical complications. Hence, the hemolytic effect of US_2 acoustic field in the presence of PPI, MPI, and LPI was compared to hemolysis of controls containing blood exposed to no ultrasound or material. This procedure is similar to the one recommended by International Organization of Standardization (ISO) 10993-4: 2002 (E) standard [28].

Citrated human whole blood pooled from multiple donors was used within 72 hours of the collection. 1 ml of blood was diluted with 7 ml of saline, transferred to custom-made polyolefin test tube (1.27 cm ID) and placed in a 37°C water bath. Each test tube was exposed either to nothing or to one of three independent prototype catheters with PPI, MPI and LPI that were positioned in the center of the tube and driven with US₂ acoustic protocol for duration of 3 hours. The diluted blood was then transferred to a microcentrifuge tube, and centrifuged at radial centrifugal force of 700 to 800 ($\times g$) for 15min. 1 ml of the resulting supernatant was withdrawn and mixed with 1 ml of Drabkin's reagent in a cuvet. After 15 minutes wait time, the absorbance at 540nm was recorded with a spectrophotometer (Genysis 10Bio, ThermoScientific, Vernon Hills). The remaining plasma was carefully withdrawn, and the volume noted. The remaining pellet of red blood cells (RBCs) was then withdrawn and transferred into a test-tube filled with a known, measured amount of distilled water. This was allowed to sit for 1 hour to ensure lysis of all RBCs. 1ml of the lysed RBC solution was transferred to a microcentrifuge tube and centrifuged at the same settings for 15min. 1 ml of the supernatant was withdrawn and diluted in 1 ml of Drabkin's reagent, and its absorbance was read at 540nm.

Hemolysis percentage was determined by subtracting control hemoglobin concentration percentage from test hemoglobin concentration percentage. The percentage of Hemoglobin concentration was calculated by accounting for any dilutions and using the following equation [29]:

$$H_b [\%] = \left(\frac{A_1}{A_1 + A_2} \right) \times 100 \quad (\text{Equation 2})$$

Whereas: A_1 ... Absorbance of the H_b in the supernatant A_2 ... Absorbance of the H_b solution after total hemolysis

Three data points were collected for each test group. Independent student's t-test was performed between the control group and the US treatment groups and the US treatment groups with each other.

RESULTS

Cavitation

At low pressure acoustic protocol, US₁, absolutely no cavitation events were observed in the presence of PPI, MPI and LPI. In the presence of high pressure acoustic protocol, US₂, the PPI still generated no cavitation events. There were, however, low level of subharmonic and broad band noise events observed in combination of US₂ and MPI. It is noteworthy to mention that subharmonic events were more dominant in comparison with broad band noise events. For LPI operated with US₂, equal levels of subharmonic and broad band noise events were recorded. The total sum of cavitation events is presented in Table 2.

Thrombolysis

Percent clot lysis with rt-PA alone (T) resulted in 13.6 ± 2.64 % loss of clot weight. Clots treated with saline regardless of PI surface texture and ultrasound protocol did not result in any statistically significant clot weight loss.

Clots treated with rt-PA and US₁ (T+US₁) resulted in percent clot lysis of 22, 21.4 and 20.6 in the presence of PPI, MPI and LPI, respectively. All the lysis enhancements relative to T group were statistically significant. Clots treated with rt-PA and US₂ (T+US₂) resulted in percent clot lysis of 24.7, 46.1 and 33.2 for PPI, MPI and LPI, respectively, with all the lysis

enhancements relative to T and T+US₁ of respective groups being statistically significant. The combination of US₂ and MPI and LPI resulted in an increase in the clot lysis rate of 2.15 and 1.61 times. A detailed summary of clot lysis is presented in Table 3.

Hemolysis

The control group included 3 blood samples incubated in water bath at 37°C for 3 hours. The resulting average hemolytic index of the control was 0.3%. The average hemolytic index of the blood samples exposed to each prototype catheter with PPI, MPI and LPI when operated with US₂ acoustic protocol was 0.4%, 0.53% and 0.49%, respectively. According to ASTM F 756-00 (2000) standard, a hemolytic index lower than 2%, is considered non-hemolytic. Hence, none of the prototype catheters operated with high pressure ultrasound caused any hemolysis for the duration of 3 hours.

The prototype with PPI operated with US₁ is similar to the device receiving FDA approval in 2005. Non-hemolytic is one of the characterization of this device hence was not repeated in this test. A summary of detailed raw data for each sample is shown in Table 4.

DISCUSSION

There is a general consensus that high-frequency (>1MHz), low-pressure ultrasound (5.2 MPa) enhances thrombolysis by means other than thermal and cavitation effects [8, 30–34]. Acoustic cavitation is defined as the generation and volumetric oscillation of small gas bubbles in an acoustic field [35]. In the absence of cavitation, it is the absorption of ultrasound energy by blood clot components that has the greatest potential for inducing bioeffects. In fact, the non-cavitation ultrasound phenomena reported to enhance rt-PA thrombolysis: 1) increase porosity of the fibrin matrix scaffolding the clot; and 2) enhanced fluid streaming within the clot; both are coupled to ultrasound absorption [30, 33–34, 36–37]. The highest peak rarefaction acoustic pressure used in this study was 2.3 MPa (US₂) which is less than reported >5.2 MPa cavitation threshold pressure in blood reported by Deng *et al.* [38]. Hence, in this study we could safely assume that absorption rather than cavitation is responsible for increased rt-PA lysis by ultrasound in the absence of the surfaces with created microcavities.

Cavitation inception from a surface requires preexisting nucleation sites that can be activated when exposed to US at an appropriate level of oscillating mechanical pressure. [35, 39–40]. Gas nuclei stabilized in the texture of a laser induced or microfringed surface can reduce the acoustic pressure required to generate cavitation at the interface, where the acoustic pressure generated by the acoustic transducer is at maximum. Since due to the geometrical spreading this pressure drops with increasing distance from the transducer surface [41]. Cavitation promoting microcavities at the interface will act as a source of submicroscopic gas bodies that can potentially grow to micron-sized nuclei at relatively low acoustic pressure thresholds, such as 1.5MPa in plasma (Figure 4) or 2.3 MPa in with rt-PA infused blood clot. At these acoustic pressure levels both cavitation and non-cavitation mechanisms could be combined and responsible for enhanced lysis rate.

It may be useful to consider the nature of the cavitation activity generated in such a system and its impact on clot lysis. When exposed to a sound field, a bubble can undergo forced, repetitive linear or nonlinear volume pulsations about an equilibrium radius, a phenomenon known loosely as stable cavitation. At sufficiently large pressure amplitudes, the same bubble can experience unstable growth followed by a rapid, energetic collapse, a process termed inertial cavitation [42]. It is less likely that stable cavitation alone can cause significant mechanical disruption of the clot. The violent collapse of an inertial bubble, however, releases a significant amount of energy in the form of an acoustic shock wave and

can induce supplemental mechanical stresses from high velocity shearing flows; both physical effects could potentiate clot fragmentation [43]. However, in this study the bubbles generated from microcavities during thrombolysis may not be necessarily free bubbles. Some or all of them could remain trapped even though they all could be contributing to the quantity of cavitation events.

The MPI demonstrated a dominant activity of subharmonics indicative of the presence of stable cavitation while the clot lysis rate increased by 2 times suggesting that clot lysis time can be reduced by half. The LPI demonstrated equal levels of subharmonics and broadband noise events, which are signatures of stable and inertial cavitation, respectively. Although the cavitation events generated by LPI were significantly higher in quantity, the resulting clot lysis rate was not as high as achieved in the presence of MPI. There is a possibility that besides the quantity of nuclei generation, their stabilization in microcavities and their release into the medium could be also influenced by the homogeneity and heterogeneity of created nucleation sites. Further investigation will be required to explain the lack of correlation among cavitation quantity and clot lysis rate observed in this study.

Cavitation is known to be associated with hemolysis. Extensive hemolysis can cause significant increase in morbidity and mortality. Hemolysis was reported to increase proportionally to the cavitation events induced by addition of ultrasound contrast agents to the blood [25–27]. Poliachek *et al.* demonstrated significant amount of hemolysis at the presence of 100 recorded cavitation events [26]. The cavitation events reported for the two cavitation promoting surfaces used in this study, MPI and LPI, were 26 and 201 for subharmonics and 3 and 217 for inertial cavitation, respectively. However, these data represent only 3.3% of cavitation events occurred during the 10 minutes sonothrombolysis and 0.19% of cavitation events occurred during 3 hours of hemolysis test. In spite of the high quantity of cavitation events, these cavitation promoting surfaces demonstrated no hemolytic effect in contrast to what was reported by other published studies. This would be another indication that the characteristics of cavitation nuclei in this study may differ from the characteristics of free bubbles in the similar acoustic field.

CONCLUSION

Introduction of a cavitation promoting surface at the boundary of acoustic source and thrombolytic drug infused thrombus can significantly enhance the thrombolysis rate and shorten required time for dissolution of the thrombus. With this approach, the acoustic pressure required for generating cavitation at high frequency ultrasound will be considerably lowered. Increased sonothrombolysis rate and lack of hemolysis are effects coupled with cavitation generated by these surfaces. An ultrasound microcatheter with cavitation promoting interface holds the potential to reduce the time to recanalization of an arterial thrombotic occlusion and improve the clinical outcome of acute ischemic stroke therapy.

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HIGHLIGHTS

To lower cavitation threshold, nucleation sites were generated on the interface of acoustic source and clot.

The Nucleation sites generated on the OD of a polyimide tube (PI) were either microfringed (MPI) or laser induced (LPI).

Both MPI and LPI showed the potential of inducing cavitation in blood clots at acoustic pressure levels as low as 2.3 MPa.

Cavitation enhanced thrombolysis rate by 2 times without any evidence of hemolysis.

A transducer with nucleation sites on outer surface could be an intra-clot treatment option for acute ischemic stroke.

Figure 1.

Microscopic images of the outer surfaces of the three sample tubes tested: a) The surface of PPI is relatively smooth, except for occasional scratches and pits. The long, straight scratches may be a result of the extrusion process used to form the tubes. b) The surface of MPI shows very coarse pattern consisting of relatively large ridges and valleys. c) This image shows the surface of LPI consisting of blind hole arrays with similar entrance diameter.

Figure 2.

a) Microsonic catheter, b) Acoustic pressure field map of the transducer mounted on the Microsonic catheter

Figure 3.
A diagram of the passive cavitation detection system

Figure 4. Ultrasound imaging of MPI before, a, and during, b, operation with US_2 in platelet poor plasma.

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Table 1

Detailed description of both acoustic protocols US₁ and US₂ operating at 1.7 MHz frequency is presented in this table. The spatial-peak pulse-average intensity, I_{SPPA}, and spatial-average acoustic pressure are the values at the external surface of catheter mounted non-focused transducers, where these values are at maximum.

Acoustic Protocol	Duty Cycle [%]	Pulse Repetition Frequency [Hz]	Temporal- Average Acoustic Power [W]	I _{SPPA} [W/cm ²]	Spatial-Average Pressure [MPa]
US ₁	8.5	30	0.3	62.46	1.37
US ₂	4	40	0.4	180.02	2.32

Table 2

The total sum of cavitation events captured by 6 snapshots (total duration of 20 seconds) during the 10 minutes sonothrombolysis procedure is presented in this table. These data present only 3.3% of cavitation events occurred during the 10 minutes sonothrombolysis.

Acoustic Protocol	Polyimide material	Peak Subharmonic events above Cavitation Threshold	Broad band noise events above Cavitation Threshold
US ₁	PPI	0	0
US ₁	MPI	0	0
US ₁	LPI	0	0
US ₂	PPI	0	0
US ₂	MPI	26	3
US ₂	LPI	201	217

Table 3

A detailed summary of percent clot lysis after sonothrombolysis by MPI and LPI in the presence and absence of US₁ and US₂ with rt-PA (T) and saline (S). Percent clot lysis with T alone resulted in 13.6 ± 2.64 % loss of clot weight. A minimum of five data points were generated for each treatment group.

Acoustic Protocol	US ₁			US ₂		
	PPI	MPI	LPI	PPI	MPI	LPI
Polyimide Surface Types						
Clot Lysis with S (%)	-3.2±2.9	-3.5±2.4	-3.6±2.2	-2.5±2.7	0.7±2.2	-4.9±2.0
Clot Lysis with T (%)	22±2.2	21.4±2.1	20.6±2.1	24.7±2.1	46.1±1.2	33.2±1.4

Table 4

A detailed summary of hemolysis data containing all three data points collected for each test group.

	Samples	Absorbance	Hemoglobin concentration (mg/ml)	Hemolytic Index (%)	Average Hemolytic Index (%)
Control	1	0.08	0.03	0.29	0.30
	2	0.08	0.02	0.27	
	3	0.09	0.03	0.34	
PPI	1	0.10	0.03	0.36	0.40
	2	0.11	0.03	0.39	
	3	0.12	0.04	0.45	
MPI	1	0.16	0.05	0.59	0.53
	2	0.12	0.04	0.43	
	3	0.15	0.05	0.55	
LPI	1	0.15	0.05	0.55	0.49
	2	0.14	0.04	0.49	
	3	0.12	0.04	0.42	